(54) Title: THE USE OF INOSITOLTRISPHOSPHATE FOR THE PREPARING OF MEDICAMENTS

(57) Abstract

The present invention relates to the use of inositoltrisphosphate (IP₃) for the preparing of a medicament with antimetastatic effects.

THE USE OF INOSITOLTRISPHOSPHATE FOR THE PREPARING OF MEDICAMENTS

The present invention relates to the use of at least one isomer of inositoltrisphosphate (IP_3) for the preparing of a medicament with antimetastatic effects.

Tumor diseases may be defined as a progressive series of genetic events which occur in a single clone of cells as a result of alterations in a limited number of specific genes i.e. the oncogenes and the tumor suppressor genes. Consistent chromosome aberrations are observed for example in lung, colon, and breast cancers. In this manner tumor diseases can be understood as a result of the accumulation of multiple genetic changes (E. Solomon et al., Science, 254, 1153-1160, 1991).

One severe type of tumor diseases are brain tumors or gliomas. In humans, gliomas account for over 60 % of primary intracranial neoplasms. Most commonly, gliomas arise from malignant transformations of astrocytes and according to histopathological criteria they are classified as astrocytoma (low grade glioma or glioblastoma, or high grade glioma). A glioblastoma is a tumor characterized by high cell density, cellular polymorphism, mitoses, necroses with palisading cells and prominent vascularisation with endothelial cell proliferation.

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Often glioblastoma is seen as the prototypical tumor when
developing new therapeutical regimes.

The progressive in vivo growth of malignant tumor cells
within tumor tissue, becomes possible only after the
formation of an adequate blood supply system within tumor
tissue. Such a system is formed by the incorporation of
existing host vessels into the tumor tissue as well as by
the creation of new tumor microvessels. The vasculature
network in human cancers is essential for the existence
of these malignant tissue systems. The tumor vasculature
network provides cancer cells with vital nutrients and
also enables the removal of waste products generated by
the cells. The vascularization of tumor tissue also in-
fluences positively the proliferation of tumor cells. For
example, tumor cells in the vicinity of blood vessels
have a greater growth rate than those tumor cells which
are located further away from blood vessels.

Angiogenesis is a central part of the process of blood
vessel formation. In normal physiological activities in-
cluding reproduction and tissue development angiogenesis
is highly regulated as it is activated for only brief
periods and then completely inhibited. In many diseases,
however, the regulation of angiogenic activity is
loosened. In some cases angiogenic activity becomes com-
pletely unregulated and its rate is exhilarated.

Angiogenic activity is central to the proliferation of
tumor cells in a human body. The often rapid development
of new blood vessels through angiogenesis provides a
highly effective means for this process. The new blood
vessels become a gateway through which tumor cells enter

the human circulatory system and eventually metastasize
into distant sites such as the liver, the lungs, bones,
etc. (J. Folkman, Y. Shing; J. Biol. Chem., 267,
10931-10934, 1992).

When a healthy cell is on the borderline of becoming a
malignant tumor cell, the tissue which surrounds the cell
must induce angiogenic vessel formation in order to
attract the nourishing vasculature which the developing
cells need for their growth. In such cases angiogenesis
is controlled by a balance between local factors which on
the one hand stimulate vasculature growth and on the
other inhibit vasculature growth. In most normal tissues,
the inhibitory influences of the local factors predominate
the angiogenic process, and cells which are derived from
such tissues generally do not stimulate angiogenesis.

In many pathological conditions, however, diseases appear
to be driven by, and spread through unregulated angio-
genesis. A primary example of such abnormal, unregulated
angiogenic activity is the proliferation and metastasis
of solid tumors.

An in vivo tumor is formed only when the host produces a
stroma and the vascular network which is required for the
survival and growth of the neoplastic cell population.

The stroma consists of (a) provisional fibrin gel matrix,
(b) new blood vessels, (c) inflammatory cells, (d)
connective tissue. Malignant growth has been shown to
depend in general on complex interactions between the
tumor and the immune- and homeostatic systems.

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The growth of solid tumors beyond microscopic clumps of
cells requires the development of a vascular network which
provides the malignant cells with nutrients and oxygen.
The additional nutrients and oxygen supplied through the
new vasculature allows dysplastic growth of the transformed
cell populations; this in turn promotes further vessel
development. Differentiation, mutation, and selection of
tumor cells occurs throughout this cycle, and the forma-
tion of three-dimensional tumors is the ultimate result
of the process. Such tumors may be locally invasive or
metastatic, or both.

A continuous remodelling of the tumor extra-cellular matrix
controlled by malignant cells which are characterized by
different degrees of biosynthesis and degradation, has
been observed during the development of primary and meta-
static tumors (Iozzo, R.V. and Cohen, I., 1993; Dvorak,
H.F. et al, 1992).

Abnormal accumulation of fluid typically accompanies
solid-, and particularly ascites tumor growth. The forma-
tion of ascites fluids is made possible by rapidly in-
creasing microvascular permeability. Observations made in
experimental animal studies as well as clinical human
studies seem to indicate that accumulation of ascites
fluid can be attributed to alterations in the permea-
bility of the vessels which form the lining for the
peritoneum and other cavities. The vessels which form the
lining for the peritoneal cavity in ascites tumor-
bearing guinea pigs, hamsters, and mice manifest signi-
ficantly greater permeability than similar vessels in
control animals. Secretion of permeability-increasing
activity appears to be a common feature in tumor tissue

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and may contribute to the abnormal accumulation of fluid
in neoplastic diseases.

All tumor tissue, solid or otherwise compact, consists of
different types of cells, never of individual cells living
in isolated environments.

Tumor diseases comprises more than one hundred different
diseases. Each of these has between two and four stages
and each can be divided into smaller, more or less critical
subtypes based on histological criteria and enzymological
and other findings. All of the different stages and
subsets are potential targets for therapeutic treatment
and combinations thereof. Such treatments include chemo-
therapy, hormone therapy, radiotherapy, cytokine therapy,
and surgery.

It is significant to note that even though the term "chemo-
therapy" is generally used to denote the application of
cytotoxic agents the term actually refers to over 30 quite
different cytotoxic agents all of which are in common
clinical use. It is also important to keep in mind that
when cytotoxic agents are administered alone or in com-
bination with one another they have extremely steep dose-
response curves. Because the dose- and response curves of
most cytotoxic drugs coincide with their toxicity- and
efficacy level curves, in therapy most of these agents
are applied at doses which are close to their maximum
level of tolerability. As a result, the harmful side
effects of cytotoxic drugs are difficult to avoid. Dis-
crepancies in individual patients's drug metabolism and
doseresponse relationship make the situation even more
problematic.

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During the past decades cytotoxic drugs have become in-
creasingly popular as a treatment for neoplastic diseases
and they have become invaluable for clinical cancer
therapy. In order to combat the heterogeneity of the
variable tumor cell metabolism of the neoplastic cell
population, many of these therapeutic agents are fre-
quently used in combination with one another. In cancer
chemotherapy such polypharmacy has become almost a rule.
Today it is also clear that many anti-cancer drugs are
essentially prodrugs which require metabolic-activation
in order to exert their selective cytotoxic effects.

Many cytotoxic agents are not very selective in terms of
their target cells i.e. they are cytotoxic also towards
normal, healthy cells.

Antineoplastic drug-therapy treatments function selectively
by targeting rapidly dividing cells. However, clinical ex-
perience shows that many curable cancers grow at a rela-
tively slow rate whereas many treatment-resistant cancers
are quite rapidly spreading.

Recent research seems to suggest that one of the most
significant effects of antineoplastic drugs is the induc-
tion of apoptosis in tumor cells. Apoptosis is a geneti-
cally encoded and activated cell death program which is
defined by specific morphological and biochemical changes
within a cell. A multitude of factors regulate apoptosis
induction; these include, for example, intracellular
mediators of signal transduction, the modulation of gene
expression by nuclear proteins, DNA replication, and cell
cycle.

Another approach for curing tumor diseases has been to
develop angiosuppresive agents. However, almost all
potential angiosuppresive agents have proven to be too
toxic for clinical use as their tolerability in humans
generally are too low.

The primary focus of cancer research is the individual
cancer cell and its metabolism. However, the symbiotic
relationship of a cancer cell and its surrounding environ-
ment has escaped critical attention.

To summarize there is a need for a new and effective anti-
tumor agent without harmful side-effects.

Ideally a new anti-tumor agent should fulfill the following
criteria: (a) it must act specifically against tumor tissue;
(b) it should have no harmful side-effects; (c) a new sub-
stance should not be a cytostatica because the aim is not
the destruction of a single tumor cell, but instead the
blocking of the tumor tissue growth mechanism; (d) it
must be able to prevent the angiogenesis of tumor tissue;
(e) it must have an antimetastatic effect.

According to the present invention it has surprisingly
become possible to use at least one isomer of inositol-
trisphosphate (IP_3), for the preparing of a medicament
with antimetastatic effects.

In preferred embodiments of the invention the medicament
is intended to be used for preventing, alleviating and
combatting tumor tissue growth and angiogensis of tumor
tissue.

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The invention relates to the use of at least one isomer
of IP$_3$ for preventing, alleviating or combatting for
example the following conditions:

Glioma, glioblastoma, sarcoma such as Kaposi sarcoma
prostate carcinoma, colon adenocarcinoma, pancreas car-
cinoma, mamma adenocarcinoma, lung carcinoma such as small
cell lung carcinoma, human histiocytic lymphoma and
melanoma.

The medicament is effective against the above mentioned
conditions but also to other conditions of tumor growth
and especially growth of solid tumors.

The medicament exerts significant antimetastatic effects
without side-effects which is very beneficial for the
patient.

From the European Patent No. 179439 a pharmaceutical com-
position comprising as a pharmaceutically active ingredient
at least one isomer of inositoltrisphosphate is known. In
said patent the effect of this pharmaceutical composition
is shown for different areas, such as platelet aggregation.

The production of IP$_3$ and the isolation of the different
isomers thereof are disclosed in the US patent No. 4.777.134.
The IP$_3$ isomers can also be produced by synthetic methods,
chemically or enzymatically, starting with e.g. inositol
and a phosphorus source. Furthermore, microbiological pro-
duction methods including hybrid DNA-techniques of IP$_3$ are
also suitable.

The structure of IP$_3$ and the different isomers thereof are
disclosed in the US patent No. 4.735.936 and the US patent
No. 4.797.390.

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It is suitable that the medicament used according to the invention exists in unit dosage form. Tablets, granules or capsules are suitable administration forms for such unit dosage. Furthermore, tablets and granules can easily be surface treated such as to provide an enteric coating to prevent an uncontrolled hydrolysis in the stomach and to bring about a desired absorption in the intestine. Other suitable administration forms are slow release and transdermal administration. A usual pharmaceutically acceptable additive, excipient and/or carrier can be included in the medicament. The tablets or granules can also contain a disintegrant which causes the tablets or the granules, respectively, to disintegrate easily in the intestine. In certain cases, especially in acute situations, it is preferable to use the unit dosage in the form of a solution for intravenous administration. In other situations suspensions comprising the compound can be preferably used as administration form.

The medicament can also consist as such of IP_3 solely without any additive, excipient or carrier.

If desired, the medicament can be free of other inositol phosphates, IP_1, IP_2, IP_4, IP_5 and IP_6. Accordingly, the mixture of IP_3 isomers can have a purity of 90-100 %, such as 93-100 % or preferably 95-100 %.

Alternatively, the medicament can consist of or comprise one or more specific IP_3 isomers, each present in substantially pure form. Thus, the different isomers can be isolated from each other in substantially pure form, which means that they have a purity of 80-100 %, such as 82-100 % or 85-100 %, preferably 90-100 %. Since the isomers can

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be produced in pure form they can be mixed in any propor-
tion, of course.

The medicament can consist of IP_3, wherein said IP_3 is
provided by at least one of IP_6, IP_5 or IP_4 and a degrada-
tive substance such as an enzyme suitable to form IP_3.

It is in most cases suitable that the IP_3-isomer or
isomers used for the preparing of the medicament according
to the invention are present in salt form in order not to
affect the mineral balance negatively. The salt should
preferably consist of a sodium, potassium, calcium zinc
or magnesium salt or a mixture of two or more of these
salts.

For the above mentioned reasons it is also an advantage
if the medicament contains a surplus or an extra addition
of at least one pharmaceutically acceptable salt of calcium,
zinc or magnesium with a mineral acid or organic acid.
This is especially valuable for elderly persons who are
often deficient in these minerals.

For administration to human patients appropriate dosages
can routinely be determined by those skilled in this art
by extension of the results obtained in animals at various
dosages. The preferred dosage for humans falls within the
range of 0.1 to 1000 mg, especially 0.1-200 mg IP_3/day/kg
body weight.

In animal experiments, no toxic effects were seen after
administration of very high doses of IP_3, 160 mg/kg body
weight by intraperitoneal injection to mice.

The medicament usually contains 0.01-1.5 g, such as
0.05-1.3 g or preferably 0.1-1 g of IP_3 per unit dosage.

The composition used according to the present invention
contains at least one, sometimes two or more of the
following substances, which correspond to the essential
IP_3-isomer or isomer mentioned above:

D-myo-inositol-1,2,6-trisphosphate of the formula
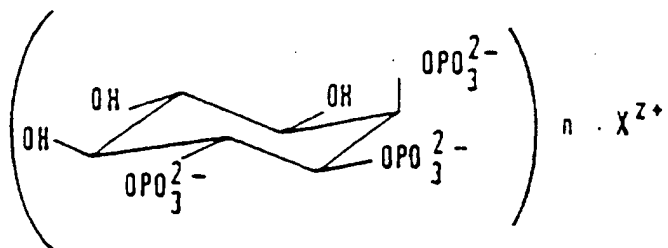
where X is hydrogen, at least one univalent, divalent or
multivalent cation, or a mixture thereof, n is the number
of ions, and z is the charge of the respective ion;

Myo-inositol-1,2,3-trisphosphate of the formula
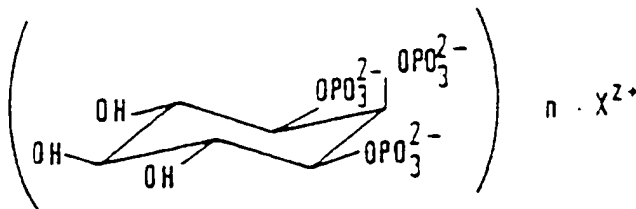
where X, n and z have the above mentioned meaning;

L-myo-inositol-1,3,4-trisphosphate of the formula

where X, n and z have the above mentioned meaning.

In each of the above formulas n ranges between 6 to 1 in-
clusive and z ranges from 1 to 6 inclusive. Preferably, n
is between 3 to 6 inclusive and z is 3, 2 or 1. Or the
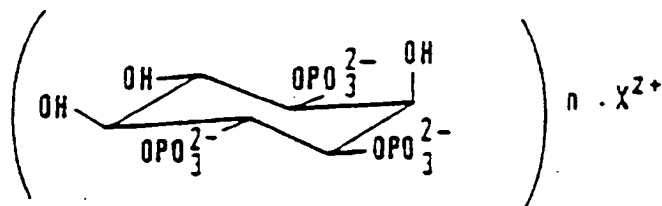above isomers D-myo-inositol-1,2,6-trisphosphate is pre-
ferred.

Other inositol trisphosphate isomers that may be utilized
in the present invention as the active IP_3 ingredient in
the composition have the structural formula

(I)
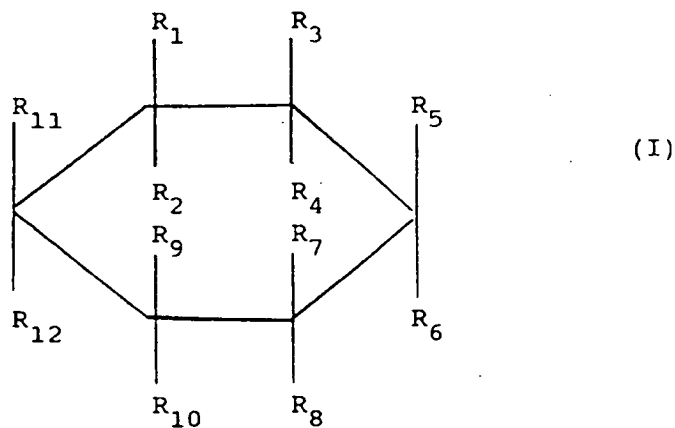
One group of inositol trisphosphate compound is defined
by the structual formula (I) where three of R_1, R_3, R_5,
R_7, R_{10} and R_{11} are hydroxyl and the remaining three are
phosphate and R_2, R_4, R_6, R_8, R_9 and R_{12} are hydrogen.

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Another group of inositol trisphosphates is defined by
the structural formula (I) where three of R_1, R_3, R_6, R_7,
R_9 and R_{12} are hydroxyl and the remaining three are phos-
phate and R_2, R_4, R_5, R_8, R_{10} and R_{11} are hydrogen.

Still another group of inositol trisphosphates is defined
by the structural formula (I) where three of R_1, R_3, R_5,
R_8, R_{10} and R_{12} are hydroxyl and the remaining three are
phosphate and R_2, R_4, R_6, R_7, R_9 and R_{11} are hydrogen.

Yet another group of inositol trisphosphates is defined
by the structural formula (I) where three of R_1, R_4, R_5,
R_8, R_9 and R_{12} are hydroxyl and the remaining three are
phosphate and R_2, R_3, R_6, R_7, R_{10} and R_{11} are hydrogen.

Still yet another group of inositol trisphosphates is de-
fined by the structural formula (I) where three of R_1,
R_3, R_6, R_8, R_9 and R_{12} are hydroxyl and the remaining
three are phosphate and R_2, R_4, R_5, R_7, R_{10} and R_{11} are
hydrogen.

Even still another group of inositol trisphosphates is
defined by the structural formula (I) where three of R_1,
R_3, R_6, R_7, R_{10} and R_{12} are hydroxyl and the remaining
three are phosphate and R_2, R_4, R_5, R_8, R_9 and R_{11} are
hydrogen.

Even yet another group of inositol trisphosphates is de-
fined by the structural formula (I) where three of R_1,
R_3, R_5, R_8, R_{10} and R_{11} are hydroxyl and the remaining
three are phosphate and R_2, R_4, R_6, R_7, R_9 and R_{12} are
hydrogen.

Finally, another group of inositol trisphosphates is defined by the structural formula (I) where three of R_1, R_3, R_5, R_7, R_9 and R_{11} are hydroxyl and the remaining three are phosphate and R_2, R_4, R_6, R_8, R_{10} and R_{12} are hydrogen.

Particular inositol trisphosphate compounds within the contemplation of the above formula include compounds having the structural formula (I) where

R_5, R_7 and R_{10} are phosphate, R_1, R_3 and R_{11} are hydroxyl and R_2, R_4, R_6, R_8, R_9 and R_{12} are hydrogen;

R_1, R_{10} and R_{11} are phosphate, R_3, R_5 and R_7 are hydroxyl and R_2, R_4, R_6, R_8, R_9 and R_{12} are hydrogen;

R_1, R_3 and R_{11} are phosphate, R_5, R_7 and R_{10} are hydroxyl and R_2, R_4, R_6, R_8, R_9 and R_{12} are hydrogen;

R_3, R_5 and R_7 are phosphate, R_1, R_{10} and R_{11} are hydroxyl and R_2, R_4, R_6, R_8, R_9 and R_{12} are hydrogen;

R_3, R_7 and R_{10} are phosphate, R_1, R_5 and R_{11} are hydroxyl and R_2, R_4, R_6, R_8, R_9 and R_{12} are hydrogen;

R_3, R_{10} and R_{11} are phosphate, R_1, R_5 and R_7 are hydroxyl and R_2, R_4, R_6, R_8, R_9 and R_{12} are hydrogen;

R_1, R_3 and R_6 are phosphate, R_7, R_9 and R_{12} are hydroxyl and R_2, R_4, R_5, R_8, R_{10} and R_{11} are hydrogen;

R_6, R_7 and R_9 are phosphate, R_1, R_3 and R_{12} are hydroxyl and R_2, R_4, R_5, R_8, R_{10} and R_{11} are hydrogen;

R_3, R_5 and R_8 are phosphate, R_1, R_{10} and R_{12} are hydroxyl and R_2, R_4, R_6, R_7, R_9 and R_{11} are hydrogen;

R_1, R_3 and R_{12} are phosphate, R_5, R_8 and R_{10} are hydroxyl and R_2, R_4, R_6, R_7, R_9 and R_{11} are hydrogen;

R_1, R_3 and R_5 are phosphate, R_8, R_{10} and R_{12} are hydroxyl and R_2, R_4, R_6, R_7, R_9 and R_{11} are hydrogen;

R_1, R_5 and R_8 are phosphate, R_3, R_{10} and R_{12} are hydroxyl and R_2, R_4, R_6, R_7, R_9 and R_{11} are hydrogen;

R_1, R_5 and R_{12} are phosphate, R_3, R_8 and R_{10} are hydroxyl and R_2, R_4, R_6, R_7, R_9 and R_{11} are hydrogen;

R_1, R_3 and R_{12} are phosphate, R_6, R_8 and R_9 are hydroxyl and R_2, R_4, R_5, R_7, R_{10} and R_{11} are hydrogen;

R_1, R_3 and R_6 are phosphate, R_7, R_{10} and R_{12} are hydroxyl and R_2, R_4, R_5, R_8, R_9 and R_{11} are hydrogen;

R_4, R_5 and R_8 are phosphate, R_1, R_9 and R_{12} are hydroxyl and R_2, R_3, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_3, R_5 and R_8 are phosphate, R_1, R_{10} and R_{11} are hydroxyl and R_2, R_4, R_6, R_7, R_9 and R_{12} are hydrogen;

R_1, R_3 and R_5 are phosphate, R_8, R_{10} and R_{11} are hydroxyl and R_2, R_4, R_6, R_7, R_9 and R_{12} are hydrogen;

R_1, R_3 and R_5 are phosphate, R_7, R_9 and R_{11} are hydroxyl and R_2, R_4, R_6, R_8, R_{10} and R_{12} are hydrogen;

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R_1, R_3 and R_{12} are phosphate, R_5, R_8 and R_9 are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_1, R_3 and R_8 are phosphate, R_5, R_9 and R_{12} are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_3, R_5 and R_{12} are phosphate, R_1, R_8 and R_9 are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_1, R_5 and R_9 are phosphate, R_3, R_8 and R_{12} are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_1, R_5 and R_{12} are phosphate, R_3, R_8 and R_9 are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_1, R_3 and R_9 are phosphate, R_5, R_8 and R_{12} are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_3, R_5 and R_9 are phosphate, R_1, R_8 and R_{12} are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_5, R_9 and R_{12} are phosphate, R_1, R_3 and R_8 are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_1, R_8 and R_9 are phosphate, R_3, R_5 and R_{12} are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_1, R_8 and R_{12} are phosphate, R_3, R_5 and R_9 are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_5, R_8 and R_{12} are phosphate, R_1, R_3 and R_9 are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_1, R_9 and R_{12} are phosphate, R_3, R_5 and R_8 are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_5, R_8 and R_9 are phosphate, R_1, R_3 and R_{12} are hydroxyl
and R_2, R_4, R_6, R, R_{10} and R_{11} are hydrogen;

R_3, R_8 and R_9 are phosphate, R_1, R_5 and R_{12} are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_3, R_9 and R_{12} are phosphate, R_1, R_5 and R_8 are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen;

R_3, R_8 and R_{12} are phosphate, R_1, R_5 and R_9 are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen; and

R_8, R_9 and R_{12} are phosphate, R_1, R_3 and R_5 are hydroxyl
and R_2, R_4, R_6, R_7, R_{10} and R_{11} are hydrogen.

The above formula describes specific isomers of inositol
trisphosphate where the inositol is selected from the
group myoinositol, cisinositol, epiinositol, alloinositol,
neoinositol, mucoinositol, chiroinositol and scylloinosi-
tol. The invention will be further explained in the
following examples without limiting it thereto. Example 1
shows the manufacturing of a solution of IP_3 for intra-
venous administration and Example 2 illustrates the
ability of IP_3 to counteract the tumor growth of glioma
tumor tissue in nude mice. Nude mice have been used
extensively for the transplantation and propagation of
human xenografts in order to design adequate in vivo ex-
periments with high correlation to effects in the human
body.

To further increase the prediction of animal experiments
for situations in the human body the severe combined
immunodeficient mouse (SCID) has been utilized as this
animal allows heterotransplantation of human tumors.
Example 3 demonstrates the antimetastatic effect of IP_3
in a prostate tumor experiment and example 4 shows the
antiangiogenetic effect of IP_3 in a lung tumor experiment.

Example 1

Solution of the sodium salt of D-myo-inositol-1,2,6-
trisphosphate (IP_3) for injection.

0.5 g of the sodium salt of IP_3 and 0.77 g sodium
chloride were dissolved in 98.73 ml of water for in-
jection to form a solution suitable for injection into a
person or an animal.

Example 2

Rat glial cells were grown in a stationary suspension
culture in a medium supplemented with 15 % heat inacti-
vated horse serum and 2.5 % heat inactivated fetal calf
serum in a 5 % CO_2 humidified atmosphere at 37°C.

The cells were harvested using trypsine solution and the
concentration of cells were measured. The cells were
centrifuged and resuspended to a concentration of 1
million cells/ml.

Twelve female specific pathogen free mice at an age of 12
weeks (weighing 20-25 g) were maintained in autoclaved
cages with filter tops in horizontal air flow cabiners in
a unit equipped with specific filters. Autoclaved bedding,

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irradiated feed and sterile water was used. The tempera-
ture and humidity was kept constant at 22 ± 2°C, 65 ± 5 %
relative humidity.

To each animal 0.1 ml of the cellsuspension was inoculated
subcutanousely in the right flank at day 0.

After five days, osmotic minipumps (ALZET) were surgi-
cally placed subcutanously into 7 animals, while 5 ani-
mals, shamoperated, served as a control group.

Each minipump contained approximately 200 μl of a solu-
tion of 1.0 g/2,5 ml of the sodium salt of 1-D-myo-ino-
sitol-1,2,6-trisphosphate (IP$_3$). The minipumps gave a
continous dosage of IP$_3$ for 14 days.

The injection of glioma cells into the animals cause a
growth of tumors. The growth of the tumors was examined
by measuring two diameters along two perpendicular axes.
The true tumor diameter was estimated as the diameter
minus 0.5 mm (which is the thickness of the skin). Tumor
area (A) was calculated by multiplying the two true dia-
meters and tumor volume was calculated by the formula:
/6 x A$^{3/2}$ x 0.67. The obtained tumor volumes are shown
in the following table:

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Tumor volume in mm^3

Day	IP$_3$-treatment	Control
0	0	0
10	115	230
13	270	580
15	620	1190
17	880	2230
20	2420	4270

The results show a very marked effect of IP$_3$ to counteract the growth of tumor tissue induced by glioma cells.

Example 3

In a procedure similar to the one described in example 2 prostate tumor growth was induced by subcutaneous in-jection of a specific cell type to mice. One group of animals received IP$_3$ via osmotic minipumps while another group of animals served as a control. The tumor growth was examined and the tumor volume is shown in the following table:

Tumor volume in mm^3

Day	IP$_3$-treatment	Control
0	0	0
7	36	52
10	62	130
17	51	481
24	95	638
28	171	1016
35	215	1132

In this example the administration of IP$_3$ reduced the growth of prostate tumor tissue in a significant way.

Example 4

Lewis lung carcinoma cells (ATCC CRL-1642) were grown as
a suspension culture in a medium with 4.5 g/l glucose
with 10 % heat inactivated bovine serum in a 5 % CO_2
humidified atmosphere at 37^OC. The cells were subcultured
by diluting cell suspensions 1:6. After harvesting by
centrifugation and resuspension, the cells were counted
in a counting chamber. The cells were resuspended with
medium to a concentration of 10 million cells/ml.

Ten male specific pathogen free mice at an age of 6 weeks
were maintained in autoclaved caves with filter tops in a
barrier unit equipped with HEPA filters. Autoclaved
bedding, irradiated feed and sterile filtered water was
used. The temperature and humidity was kept constant
(22 ± 2^OC, 65 ± 5 % RH).

To each animal 0.1 ml of the cell suspension was inocu-
lated subcutanousely in the right flank at day 0. After
15 days, osmotic minipumps (ALZET) were surgically placed
subcutaneously into 5 animals while another 5 animals,
sham operated, served as a control group.

Each minipump contained 226 μl of a solution of 0.4 g/ml
of the sodium salt of 1-D-myo-inositol-1.2.6-trisphosphate
(IP_3). The minipumps gave a continous dosage of IP_3 for 6
days.

The injection of lung carcinoma cells cause a formation
of tumors and angiogenesis in lung tissue. The weight of
the lungs after the experiment is considered as a mea-
surement of the amount of tumor tissue which is formed.

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In the sham operated animals, the mean lung weight was
0.312 g while the animals treated with IP_3 has a mean
lung weight of 0.198 g. Furthermore, histological exa-
mination showed lower angiogenesis in the animals treated
with IP_3. Thus the treatment with IP_3 reduces the tumor
tissue and diminishes angiogenesis.

CLAIMS

1. The use of at least one isomer of inositoltrisphos-
    phate (IP_3) for the preparing of a medicament with
    antimetastatic effects.

2. The use of at least one isomer of inositoltrisphos-
    phate (IP_3) for the preparing of a medicament for
    preventing, alleviating or combatting tumor tissue
    growth and angiogenesis of tumor tissue.

3. The use according to claim 1 for treatment of glioma.

4. The use according to anyone of claims 1-3 wherein
    said isomer of inositoltrisphosphate is in salt form.

5. The use according to claim 4 wherein said inositol-
    trisphosphate is a salt of sodium, potassium, calcium
    or zinc.

6. The use according to anyone of claims 1-5 wherein the
    medicament is in unit dosage forms comprising tablets,
    granules, capsules, solutions or suspensions.

7. The use according to anyone of claims 1-6 wherein
    said inositoltrisphosphate is D-myo-inositol-1,2,6-
    trisphosphate.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 31/66, C07F 9/117

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K, C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4952396 A (ROBERT SABIN ET AL.), 28 August 1990 (28.08.90), SEE ESPECIALLY CLAIM 7	1-7
	--	
X	US 4590060 A (UDO EHRENFELD), 20 May 1986 (20.05.86), SEE ESPECIALLY COLUMN 12 LINES 22-27	1-7
	--	

☐ Further documents are listed in the continuation of Box C.   ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" erlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 April 1996	**16.04.96**

Name and mailing address of the ISA/	Authorized officer
Swedish Patent Office Box 5055, S-102 42 STOCKHOLM	GÖRAN KARLSSON
Facsimile No. +46 8 666 02 86	Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 95/01585

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US-A- 4590060	20/05/86	AU-B,B- 560178	02/04/87
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